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Clive A. Slaughter

GRU-UGA Medical Partnership, Athens, GA

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NUCLEIC ACID SEQUENCING AND CHARACTERIZATION

Huddleston J, Ranade S, Malig M, Antonacci F, Chaisson M, Hon L, Sudmant P H, Graves T A, Alkan C, Dennis M Y, Wilson R K, Turner S W, Korlach J, Eichler E E. Reconstructing complex regions of genomes using long-read sequencing technology. *Genome Research* 24;2014:688–696.

Kuleshov V, Xie D, Chen R, Pushkarev D, Ma Z, Blauwkamp T, Kertesz M, Snyder M. Whole-genome haplotyping using long reads and statistical methods. *Nature Biotechnology* 32;2014:261–266.

Adey A, Burton J N, Kitzman J O, Hiatt J B, Lewis A P, Martin B K, Qiu R, Lee C, Shendure J. The haplotype-resolved genome and epigenome of the aneuploid HeLa cancer cell line. *Nature* 500;2013:207–211.

Next-generation sequencing techniques have now largely replaced the slower, more laborious, more costly capillary-based sequencing approaches. The short reads available from next-generation procedures, however, present difficulties for de novo genome assembly, especially across regions of repetitive sequence or recent duplication. Three papers highlight applications in which recent innovations in acquisition of long sequence reads have been essential in advancing knowledge of genome structure. Huddleston et al. use single-molecule, real-time sequencing technology from Pacific Biosystems to resolve the structure of a poorly assembled, 766-kbp, duplicated region of the chimpanzee genome and present evidence that the approach is suitable for targeted sequencing of such difficult regions to upgrade existing genomes to a higher-quality, finished state. Kuleshov et al. use a statistically aided method for recovering haplotype information from long DNA fragments analyzed by short read sequencing. They phase 99% of single-nucleotide variants from three human genomes into haplotype blocks of 0.2–1.0-Mbp length and apply this method to determining allele-specific methylation patterns. Adey et al. conduct haplotype-resolved, whole-genome sequencing of HeLa cells, a task rendered challenging by extensive aneuploidy, using approaches that include mate-pair and long-read sequencing and sequencing of pools of fosmid clones. They reconstruct an amplified, highly rearranged region of chromosome 8, at which integration of the human papilloma virus type 18 genome occurred, probably initiating the cancer from which the HeLa cell line was originally derived in 1951. The work is published with thanks to Henrietta Lacks, now deceased, and to her surviving family members, whose approval permitted publication. The work honors their contribution to scientific progress and human health.

doi: 10.7171/jbt.14-2502-004

Fu G K, Wilhelmy J, Stern D, Fan H C, Fodor S P A. Digital encoding of cellular mRNAs enabling precise and absolute gene expression measurement by single-molecule counting. *Analytical Chemistry* 86;2014:2867–2870.

Islam S, Zeisel A, Joost S, La Manno G, Zajac P, Kasper M, Lonnerberg P, Linnarsson S. Quantitative single-cell RNA-seq with unique molecular identifiers. *Nature Methods* 11;2014:163–166.

RNA sequencing (RNA-Seq) has become a widely accepted approach to transcriptome profiling because of its high coverage and single-nucleotide resolution. Quantification of mRNA species is usually based on number of reads, but this measure is susceptible to severe bias and nonuniformity of response that must be corrected or compensated for. Problems include the inefficiency of the cDNA synthesis reaction used to create the library to be sequenced and amplification bias that introduces inaccu-

racy. An effective way to overcome these difficulties is to incorporate unique molecular identifiers (barcodes) randomly into individual mRNA molecules within the population during the initial cDNA synthesis step. The complexity of the barcode repertoire is adjusted to ensure different labeling of each mRNA molecule of the same species so that each can later be counted individually by deep sequencing. In this way, absolute numbers of each mRNA species are measured despite amplification bias, provided sufficient sequencing depth to ensure detection of rare species is achieved. Fu et al. and Islam et al. independently apply this methodology to transcriptome characterization at the level of individual cells. Both groups demonstrate accurate, precise measurements for the small quantities of mRNA available from single cells. The methodology is an easy, single-tube assay requiring only standard thermal cyclers and PCR reagents. It promises to be effective for investigating heterogeneous and oscillatory gene expression in cell populations of otherwise homogeneous type.

MACROMOLECULAR SYNTHESIS AND SYNTHETIC BIOLOGY

Annaluru N, Muller H, Mitchell L A, Ramalingam S, Stracquadanio G, Richardson S M, Dymond J S, Kuang Z, Scheifele L Z, Cooper E M, Cai Y, Zeller K, Agmon N, Han J S, Hadjithomas M, Tullman J, Caravelli K, Cirelli K, Guo Z, London V, Yeluru A, Murugan S, Kandavelou K, Agier N, Fischer G, Yang K, Martin J A, Bilgel M, Bohutskyi P, Boulier K M, Capaldo B J, Chang J, Charoen K, Choi W J, Deng P, DiCarlo J E, Doong J, Dunn J, Feinberg J I, Fernandez C, Floria C E, Gladowski D, Hadidi P, Ishizuka I, Jabbari J, Lau C Y L, Lee P A, Li S, Lin D, Linder M E, Ling J, Liu J, Liu J, London M, Ma H, Mao J, McDade J E, McMillan A, Moore A M, Oh W C, Ouyang Y, Patel R, Paul M, Paulsen L C, Qiu J, Rhee A, Rubashkin M G, Soh I Y, Sotuyo N E, Srinivas V, Suarez A, Wong A, Wong R, Xie W R, Xu Y, Yu A T, Koszul R, Bader J S, Boeke J D, Chandrasegaran S. Total synthesis of a functional designer eukaryotic chromosome. *Science* 344;2014:55–58.

A fully synthetic version of *Saccharomyces cerevisiae* chromosome III (synIII) is announced in this paper. Chromosome III is the third-smallest in yeast, consisting of 316,617 bp. The synthetic version, containing 272,871 bp, represents a designer chromosome that incorporates several features of experimental importance. Introns and subtelomeric regions are omitted. TAG stop-codons are replaced by TAA stop-codons to free the TAG codon for encoding artificial amino acids later in the study. Transposons and 11 tRNA genes (sites of cohesion loading) are omitted to promote genomic stability. Ninety-eight loxP sites are introduced to enable genome scrambling: a modified version of the Cre recombinase enzyme randomly inverts or deletes DNA lying between pairs of loxP sites upon activation by estradiol, thereby enabling controlled amounts of genome scrambling to occur. PCR tags are introduced to distinguish the sequence of synIII from natural yeast chromosome III. The synthetic chromosome was assembled in three stages: firstly, 60- to 79-mer oligonucleotides were assembled into 750-bp building blocks by PCR; secondly, overlapping building blocks were assembled into 2–4 kb minichunks; and thirdly, adjacent minichunks, which overlapped one another by one building block, were introduced into yeast cells in successive rounds to be assembled by homologous recombination in vivo. The assembly process is shown to be highly accurate. synIII is shown to be fully functional in yeast cells. The synthetic chromosome is envisioned to be a test-bed for exploring synthetic lethal interactions between gene knockouts. The present study represents a milestone in synthetic biology and is expected to be the beginning of a project to replace all yeast chromosomes with synthetic versions to create a completely designer eukaryote.

Xu Y, Cai C, Chandarajoti K, Hsieh P-H, Li L, Pham T Q, Sparkenbaugh E M, Sheng J, Key N S, Pawlinski R, Harris E N, Linhardt R J, Liu J. Homogeneous low-molecular-weight heparins with reversible anticoagulant activity. *Nature Chemical Biology* 10;2014:248–250.

Low-molecular-weight heparins (LMWHs) are heterogeneous mixtures made by depolymerizing heparin that has been isolated from porcine intestine. LMWHs have become important anticoagulant drugs, but their production depends on a long supply chain that has proven susceptible to contamination and adulteration. The present paper describes the chemoenzymic synthesis of defined, synthetic LMWHs. The syntheses are accomplished with a carefully designed sequence of steps catalyzed by recombinant enzymes. One of the products obtained combines the clinically important features of high anticoagulant activity and sensitivity to inactivation with protamine. It is therefore hoped that the methods described here will contribute to the modernization of LMWH therapeutic agents.

SMALL MOLECULE ANALYSIS AND METABOLOMICS

Dutkiewicz E P, Lin J-D, Tseng T-W, Wang Y-S, Urban P L. Hydrogel micropatches for sampling and profiling skin metabolites. *Analytical Chemistry* 86;2014:2337–2344.

Diverse metabolites and a variety of drugs that includes illicit doping agents, narcotics, and cannabinoids are excreted in sweat. A standard method for diagnosis of cystic fibrosis involves quantification of Cl^- in sweat stimulated by pilocarpine iontophoresis. Yet, analysis of sweat has been used in very few other applications, in part, because of the difficulty of collecting sweat in a manner compatible

with modern analytical methods. The present paper describes a method for collecting and profiling sweat quickly and noninvasively. An agarose hydrogel patch is applied to the skin for 10 min and is then subjected to mass spectrometric analysis using desorption electrospray ionization (DESI). Metabolites identified by DESI include lactic acid, urocanic acid, and various amino acids. It is hoped that this methodology will provide the basis for semiquantitative assays in clinical diagnosis and forensics.

PROTEINS—PURIFICATION AND CHARACTERIZATION

Zhao H, Mayer M L, Schuck P. Analysis of protein interactions with picomolar binding affinity by fluorescence-detected sedimentation velocity. *Analytical Chemistry* 86; 2014:3181–3187.

Very high-affinity, protein-binding interactions with equilibrium dissociation constants (K_D) in the low picomolar range are commonly encountered in antibody-antigen binding. The measurement of the stoichiometry and free energy of such interactions requires quantitative monitoring of the interacting proteins at low picomolar concentration. Zhao et al. demonstrate how velocity sedimentation in an analytical ultracentrifuge equipped for fluorescence detection can combine the necessary sensitivity, precision, and accuracy for such measurements. Among the requirements are: adjusting photomultiplier voltage and laser power to give the best signal-to-noise ratio; choosing a carrier protein that does not interact with the proteins under study to prevent absorptive losses; including data acquired well beyond the time it takes to sediment the proteins of interest to regularize correctly peaks of lowest *s* value in the sedimentation coefficient distribution [*c*(s)]; recording the position of the air-water meniscus (an essential parameter in the calculation of *s* values) from an offset observed in the fluorescence signal where the meniscus is located; and including as many scans as possible to help distinguish signal from noise. By extending the sensitivity of the methodology in these ways, the authors are able to measure K_D values, two orders of magnitude lower than previously achieved by sedimentation velocity methods.

Politis A, Stengel F, Hall Z, Hernandez H, Leitner A, Walzthoeni T, Robinson C V, Aebersold R. A mass spectrometry-based hybrid method for structural modeling of protein complexes. *Nature Methods* 11;2014:403–406.

This study demonstrates the power of combining three different mass spectrometry (MS)-based methods to elucidate the architecture of macromolecular assemblies. The methods are: label-free, quantitative analysis of tryptic peptide mixtures by liquid chromatography (LC)-MS/MS for determining the composition and relative abundance of protein subunits in the assembly; native MS of intact complexes and their subcomplexes for acquiring information about overall stoichiometry and protein–protein interactions; and ion mobility spectrometry coupled with MS for elucidating protein architecture and dynamics by measurement of collisional cross-sections. The authors also use chemical cross-linking to identify subunit interfaces. They illustrate the application of this combination of techniques by study of three well-characterized protein complexes: methane monooxygenase hydroxylase from *Methylococcus capsulatus*, toluene/o-xylene monooxygenase hydroxylase from *Pseudomonas stutzeri*, and urease from *Klebsiella aerogenes*. They then tackle the architecture and assembly of the proteasome "lid" complex. It is hoped that such combined mass spectrometric approaches will contribute to knowledge of protein assemblies, especially in the range of 50–300 kDa, which is particularly challenging for electron microscopy.

PROTEOMICS

Gerster S, Kwon T, Ludwig C, Matondo M, Vogel C, Marcotte E M, Aebersold R, Bühlmann P. Statistical approach to protein quantification. *Molecular & Cellular Proteomics* 13;2014:666–677.

Gerster et al. provide a statistical framework for computing protein abundance from mass spectrometric measurements of peptide quantity. The framework allows information from peptides shared by two or more proteins to be included in the computation. This feature is particularly important, as such shared peptides are common in higher eukaryotes, and it is often difficult to observe sufficient numbers of unique peptides/protein for quantification. The proposed model also allows for propagation of uncertainty in peptide identification to the protein level and provides opportunity to reassess peptide measurements based on estimated protein concentrations, classifying extreme abundance measurements as regular data points or as actual outliers.

Burgess M W, Keshishian H, Mani D R, Gillette M A, Carr S A. Simplified and efficient quantification of low-abundance proteins at very high multiplex via targeted mass Spectrometry. *Molecular & Cellular Proteomics* 13;2014: 1137–1149.

In attempting to cope with the complexity of the plasma protein mixture in LC-MS/MS analysis of tryptic peptides, even after depletion of the most abundant plasma proteins before trypsin digestion, tryptic digests are often fractionated into six to eight pools that are analyzed separately by LC-MS/MS. This practice decreases throughput and increases the complexity of subsequent data analysis. Burgess et al. here demonstrate that improving the quality

of on-line LC separation of the peptide mixture obviates the need for such prefractionation. To improve chromatographic performance, they use long (>30 cm) columns, packed with a stationary phase of 1.9 μm particle size, and apply heat during separation. These practices result in a fourfold improvement in median limit-of-quantitation values and increase assay precision. They yield an approximately threefold increase in analysis throughput compared with subfractionation procedures. The penalty in loss of sensitivity is less than twofold. The authors take advantage of this methodology to develop a very highly multiplexed assay, in which multiple reaction monitoring of 2400 transitions (with retention time scheduling) is used to monitor 400 unlabeled and heavy isotope-labeled peptide pairs in a single analysis.

FUNCTIONAL GENOMICS AND PROTEOMICS

Murtha M, Tokcaer-Keskin Z, Tang Z, Strino F, Chen X, Wang Y, Xi X, Basilico C, Brown S, Bonneau R, Kluger Y, Dailey L. FIREWACh: high-throughput functional detection of transcriptional regulatory modules in mammalian cells. *Nature Methods* 11;2014:559–565.

Dickel D E, Zhu Y, Nord A S, Wylie J N, Akiyama J A, Afzal V, Plajzer-Frick I, Kirkpatrick A, Gottgens B, Bruneau B G, Visel A, Pennacchio L A. Function-based identification of mammalian enhancers using site-specific integration. *Nature Methods* 11;2014:566–571.

The identification of functionally active regulatory sequences that control the transcription of genes on the same chromosome (i.e., *cis* regulatory enhancers) is made difficult by the short length of the transcription factor-binding elements (often 6–8 bp). The sequence of such binding elements is insufficient on its own to identify enhancers accurately. The highly variable distance between enhancers and the genes they control also contributes to the difficulty in identifying enhancers. Two groups here present new, functional assays for discovery of enhancer elements. Murtha et al. enrich accessible chromatin, where enhancers are located, by digesting nuclei with frequently cutting nucleases. They then clone fragments of the released DNA into a lentiviral vector upstream of a GFP reporter gene. Viruses carrying different inserts are transduced into a cell line, and cells carrying an active enhancer are isolated by FACS. The putative enhancers are identified by sequencing and mapping the sequences to the genome of origin. Dickel et al. seek to identify enhancers of specific genes cloned together into bacterial artificial chromosomes (BACs). They fragment the BAC and clone the fragments upstream of a yellow fluorescent protein reporter gene and then integrate these clones into a reproducible chromosomal context, the *hypoxanthine-guanine phosphoribosyltransferase* locus of mouse embryonic stem cells, in vitro-differentiated cardiomyocytes, or neural progenitor cells. Like Murtha et al., they then use FACS and sequencing to identify enhancer candidates. Although these methods are not intended for comprehensive discovery of enhancers, the incorporation of functional assays contributes to a high rate of correct identification of active enhancer elements.

CELL BIOLOGY AND TISSUE ENGINEERING

Zhu S, Rezvani M, Harbell J, Mattis A N, Wolfe A R, Benet L Z, Willenbring H, Ding S. Mouse liver repopulation with hepatocytes generated from human fibroblasts. *Nature* 508;2014:93–97.

Although it has been hoped that induced pluripotent stem cells (iPSCs) will provide differentiated hepatocytes for research and therapy for liver diseases, hepatocytes derived from iPSCs have not exhibited the capacity for replication that would enable them to populate an experimental animal, such as a mouse. This disappointing outcome has therefore also cast doubt on the potential of iPSCs for liver cell therapy. Zhu et al. here report conditions under which human fibroblasts convert to a hepatocyte phenotype via an induced multipotent progenitor cell (iMPC) state rather than an iPSC state and show that the resulting hepatocytes retain the ability to proliferate in vivo. When transplanted into an immune-deficient mouse, these induced hepatocytes acquire levels of hepatocyte function similar to transplanted adult human hepatocytes, which proliferate extensively. The iMPC-derived hepatocytes do not form tumors. These results provide encouragement that autologous liver cell therapy will eventually become possible.

Jaitin D A, Kenigsberg E, Keren-Shaul H, Elefant N, Paul F, Zaretsky I, Mildner A, Cohen N, Jung S, Tanay A, Amit I. Massively parallel single-cell RNA-Seq for marker-free decomposition of tissues into cell types. *Science* 343; 2014:776–779.

Jaitin et al. present an automated pipeline for massively parallel, single-cell RNA-seq. The method is based on FACS of single cells into 384-well plates and molecular barcoding to enable molecule counting. The authors seek to classify cells in complex tissues on the basis of RNA expression profile alone. With the application of their pipeline to analysis of splenic tissue, they distinguish, by hierarchical clustering of transcriptional profiles, five main classes of cells. By matching these classes to existing transcriptional profiles of cell types defined on the basis of conventional cell-surface markers, the authors identify the classes as B cells, NK cells, macrophages, monocytes, and dendritic cells. This achievement validates the use of classification algorithms to classify cell types. The data further

reveal additional cell types, as well as various subtypes of dendritic cells, whose transcriptional profiles suggest a degree of heterogeneity and pathway activity that does not fit easily with a preprogrammed hierarchy of cell subtypes. It is hoped that the origins and relationships between these cellular subtypes will emerge from further study of single-cell transcriptional profiles.

VACCINES AND IMMUNOTHERAPY

Zhou P, Shaffer D R, Alvarez Arias D A, Nakazaki Y, Pos W, Torres A J, Cremasco V, Dougan S K, Cowley G S, Elpek K, Brogdon J, Lamb J, Turley S J, Ploegh H L, Root D E, Love J C, Dranoff G, Hacohen N, Cantor H, Wucherpfennig K W. In vivo discovery of immunotherapy targets in the tumour microenvironment. *Nature* 506;2014:52–57.

Zhou et al. describe a new screening method to identify genes that modulate the function of T lymphocytes in vivo. The method uses RNA interference (RNAi) by short hairpin RNA (shRNA) to produce loss of function in target genes. RNAi-based functional screening has most commonly been used in vitro. The present study breaks new ground in its deployment of new methods for screening in vivo. The authors' application of the methodology in tumor immunotherapy is itself of special, topical interest. They seek genes that when knocked down in T killer ($CD8^+$) cells, cause increased reactivity of the killer cells toward tumors. They target 255 genes whose expression is associated with T cell exhaustion or anergy—a state that is believed to contribute to cancer growth—and 1307 genes encoding kinases and phosphatases of cell signaling pathways that might be involved in T cell responses to tolerance-inducing signals from cancer cells or regulatory T cells. They deliver shRNAs via lentiviral infection to $CD8^+$ T cells carrying the OT-1 T cell receptor. This receptor activates T cells in response to target cells expressing ovalbumin (Ova). They implant T cells stably expressing shRNA into mice that have melanomas that expresses Ova. After 7 days, they purify T cells that infiltrate the tumors or T cells from irrelevant lymphoid organs and identify by deep sequencing the shRNAs specifically associated with T cells that infiltrate the tumors. These are the cells that have putative anti-tumor reactivity. The authors identify a gene for a regulatory subunit of the protein phosphatase 2A phosphatase family and show that knockdown of this gene inhibits T cell apoptosis and enhances T cell proliferation and cytokine production. The genetic assay approach is hoped to find application in studies of the control of diverse immune cell functions in vivo.

Lee E C, Liang Q, Ali H, Bayliss L, Beasley A, Bloomfield-Gerdes T, Bonoli L, Brown R, Campbell J, Carpenter A, Chalk S, Davis A, England N, Fane-Dremucheva A, Franz B, Germaschewski V, Holmes H, Holmes S, Kirby I, Kosmac M, Legent A, Lui H, Manin A, O'Leary S, Paterson J, Sciarrillo R, Speak A, Spensberger D, Tuffery L, Waddell N, Wang W, Wells S, Wong V, Wood A, Owen M J, Friedrich G A, Bradley A. Complete humanization of the mouse immunoglobulin loci enables efficient therapeutic antibody discovery. *Nature Biotechnology* 32;2014:356–363.

A mouse strain expressing the full complement of human immunoglobulin variable regions with mouse constant regions is described in this paper. The authors inserted the complete set of human heavy chain, λ light chain, and κ light chain variable regions into the corresponding loci of mouse embryonic stem cells using a procedure called sequential recombinase-mediated cassette exchange. They silenced the endogenous mouse variable region genes using a large chromosomal inversion. The resulting mice are viable, fertile, and mount a repertoire of strong antibody responses similar to humans. The mice produce chimeric antibodies with human variable regions and mouse constant regions. These antibodies can subsequently be humanized by established procedures. The mouse strain will be made available to the academic community through a public access program (http://www.kymabaccess.org/). It provides a robust platform for discovery of new, therapeutic human monoclonal antibodies and is hoped also to provide an animal model for the human antibody response that will be appropriate for vaccine design.

MICROFLUIDICS

Xu S, Zhang Y, Jia L, Mathewson K E, Jang K-I, Kim J, Fu H, Huang X, Chava P, Wang R, Bhole S, Wang L, Na Y J, Guan Y, Flavin M, Han Z, Huang Y, Rogers J A. Soft microfluidic assemblies of sensors, circuits, and radios for the skin. *Science* 344;2014:70–74.

There is broad scope for development of devices that can be mounted on the skin for physiological monitoring and intervention in the treatment of medical conditions. However, the components from which such devices must be fabricated, including electronic chips, are generally hard, planar objects that do not deform as the skin stretches with movement. Xu et al. describe how devices incorporating such components may be fabricated to conform to the soft, textured, curved, dynamic surface of the skin. Each component is bonded to support posts on a thin, elastomeric substrate of silicone that has a modulus (stiffness) and elasticity matching skin. Another silicone sheet is bonded on top as a "superstrate", and the space between is filled by injecting a high molecular-weight silicone oligomer that provides high electrical resistivity to eliminate cross-talk between the electronic components, high dielectric strength to avoid electric breakdown, moderate viscosity to enhance impact resistance, hydrophobic character to expel

moisture, low chemical reactivity to resist corrosion, and optical transparency to allow inspection of components within. Components within the device are connected together by a free-floating, layered network of serpentine-shaped interconnects that can buckle and deform with stretching. Configurations are designed to avoid high principle strains, entanglements between interconnects, and collisions between components. Devices are fabricated to include modules for wireless power supply via resonant-inductive energy transfer and radiofrequency transmission of acquired data. The substrate softly laminates onto the surface of the skin. This design technology has been used to fabricate devices for recording electrocardiograms, electromyograms, electrooculograms, and electroencephalograms and has incorporated motion sensors (triaxial accelerometer) and thermal sensors. Many applications can be envisaged, including physiologic monitoring in neonatal intensive care units, continuous assessment of responses to pharmaceuticals administered at home, training in sports, and tracking in sleep-apnea studies.

POLICY

Carr S A, Abbatiello S E, Ackermann B L, Borchers C, Domon B, Deutsch E W, Grant R P, Hoofnagle A N, Ḧuttenhain R, Koomen J M, Liebler D C, Liu T, MacLean B, Mani D, Mansfield E, Neubert H, Paulovich A G, Reiter L, Vitek O, Aebersold R, Anderson L, Bethem R, Blonder J, Boja E, Botelho J, Boyne M, Bradshaw R A, Burlingame A L, Chan D, Keshishian H, Kuhn E, Kinsinger C, Lee J S H, Lee S-W, Moritz R, Oses-Prieto J, Rifai N, Ritchie J, Rodriguez H, Srinivas P R, Townsend R R, Van Eyk J, Whiteley G, Wiita A, Weintraub S. Targeted peptide measurements in biology and medicine: best practices for mass spectrometry-based assay development using a fit-for-purpose approach. *Molecular & Cellular Proteomics* 13;2014: 907–917.

The authors summarize recommendations for the criteria to be applied in judging whether targeted mass spectrometric assays for measuring peptide abundance work as intended to meet the goals for which the assays were designed. The recommendations were formulated in a workshop held at the U.S. National Institutes of Health, attended by scientists from in vitro diagnostic companies, clinical laboratories, labs specializing in quantitative assay development for candidate biomarkers, and biology-focused labs. The most commonly used approach for quantification is multiple-reaction monitoring, usually on triple quadrupole mass spectrometers. Attendees considered how to apply the principles, long-established for small molecule quantification, to the new arena of peptide quantification. Contributors described what criteria they adopt for determining whether an assay meets required levels of performance and what information must be supplied by authors to allow reviewers and readers to understand clearly what procedures they performed and to evaluate the reliability of the quantification measurements reported. *Molecular & Cellular Proteomics* intends to develop guidelines for authors based on these recommendations to fulfill a need in quantitative proteomics that parallels that which faced the community in assessing the reliability of work in discovery proteomics before 2004.